

Stable isotopic detection of ammonium and nitrate assimilation by phytoplankton in the Waquoit Bay estuarine system

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Abstract

We measured concentration and $\delta^{15}\text{N}$ of chlorophyll *a* (Chl *a*), NO_3^- , and NH_4^+ along a salinity gradient in Childs River, Massachusetts, in winter, spring, and summer. We used the $\delta^{15}\text{N}$ of Chl *a* as a proxy for the phytoplankton $\delta^{15}\text{N}$ to minimize potential ambiguities from other material in seston. NO_3^- concentration ranged from 0 to 50 $\mu\text{mol L}^{-1}$ and NH_4^+ from 0 to 8 $\mu\text{mol L}^{-1}$; both forms decreased with increasing salinity. NO_3^- concentration was generally higher than NH_4^+ . Chl *a* concentrations ranged between 1 and 15 mg m^{-3} in winter-spring and had a summer midestuarine peak of 95 mg m^{-3} . The $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ ranged from -10‰ to $+7\text{‰}$ and -3‰ to $+13\text{‰}$, respectively, and decreased approximately linearly with increasing salinity. The $\delta^{15}\text{N}$ of NO_3^- reflected the predominance of groundwater as the source of NO_3^- to the estuary, whereas the $\delta^{15}\text{N}$ of NH_4^+ indicated that regeneration was the main NH_4^+ source. Throughout the estuary, NO_3^- was isotopically lighter than NH_4^+ . Phytoplankton $\delta^{15}\text{N}$ increased from winter to summer and was relatively invariant with salinity, in contrast to the $\delta^{15}\text{N}$ of dissolved inorganic nitrogen. A comparison of the $\delta^{15}\text{N}$ of phytoplankton, NO_3^- , and NH_4^+ indicated that phytoplankton in Childs River derived 53% to 97% of their N from NH_4^+ . Phytoplankton acquired their stable nitrogen isotopic ratio upstream, then maintained that ratio during downstream transport. The fractionation factor for phytoplankton NH_4^+ uptake was $+4.0\text{‰} \pm 0.6\text{‰}$, which was in the lower range of other estimates, indicating that phytoplankton might have been N limited.

Phytoplankton take up ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and dissolved organic nitrogen (McCarthy 1980; Bronk and Glibert 1993). Although some studies have shown that NO_3^- is the preferred nitrogen (N) source for some phytoplankton species, the general consensus is that NH_4^+ is preferred (Glibert et al. 1982; Dortch 1990; L'Helguen et al. 1996). Laboratory measurements have found that when both NH_4^+ and NO_3^- are available, phytoplankton usually prefer NH_4^+ , and the presence of NH_4^+ could inhibit NO_3^- uptake, even at low

NH_4^+ concentrations ($<1 \mu\text{mol L}^{-1}$; see review by Dortch 1990).

Field measurements have shown that NH_4^+ may provide the majority of N used by phytoplankton, although NH_4^+ makes up a small portion of the dissolved inorganic nitrogen (DIN) pool. Several studies that use ^{15}N -labeled DIN sources and bottle incubations showed that the majority (up to 95%) of DIN assimilated by the plankton community was assimilated as NH_4^+ , although NO_3^- dominated the DIN pool (Pennock 1987; Kocum et al. 2002). Also on the basis of ^{15}N additions, Horrigan et al. (1990a) showed that Chesapeake Bay phytoplankton preferred NH_4^+ to NO_3^- year round, but that NO_2^- was equally preferred in spring.

The mechanism responsible for NH_4^+ preference is not fully understood, although it is known that NO_3^- must be reduced, requiring energy, before N can be used in protein synthesis. The extra energetic cost associated with the use of NO_3^- compared with NH_4^+ (Syrett 1981) likely plays a role in phytoplankton NH_4^+ preference. The presence of NH_4^+ in cell cultures depresses the abundance of transcripts of nitrate transporter genes (Hildebrand and Dahlin 2000), although the direct mechanism by which this occurs has still not been described.

Eutrophication of coastal and estuarine waterbodies is increasing (Cloern 2001) and is usually associated with increased availability of DIN to primary producers. Human influences that lead to eutrophication generally result in increased delivery of NO_3^- to these systems (Vitousek et al. 1997; Cloern 2001), which could markedly change the ratio of NO_3^- to NH_4^+ available in water for

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Acknowledgments

Stable isotopic analyses of Chl *a* were done by C. Johnson at the Woods Hole Oceanographic Institution (WHOI) GC-IRMS facility. Stable isotopic analyses of water column nitrate and ammonium were done by D. Harris at the University of California Davis Stable Isotope Facility. We thank Jim McClelland for help interpreting DIN isotopic data and Bruce Peterson for suggestions to improve the manuscript. We also thank Nicolas Savoye and an anonymous reviewer for their thorough comments, which greatly improved this manuscript.

This research was supported by a National Oceanic and Atmospheric Administration–National Estuarine Research Reserve (NOAA-NERR) Graduate Fellowship (NA17OR1198), an Environmental Protection Agency Science to Achieve Results Graduate Fellowship (U-91613501-0), and a Boston University Palmer-McLeod Fellowship to J.K.Y.; an NOAA-NERR Graduate Fellowship (NA07OR0274) to G.T.; and a WHOI Sea Grant (NA16RG2273).

phytoplankton. These alterations make the question of which form of N is assimilated by phytoplankton in nature increasingly salient because phytoplankton should prefer NH_4^+ but are often presented with a predominance of NO_3^- . This further poses the question of whether the preference for NH_4^+ might be less marked as the concentration of NO_3^- increases.

We wanted to re-examine phytoplankton N preference using methods involving a minimum of manipulation. Much of our understanding of phytoplankton N preference is based on laboratory cultures or shipboard incubations, which can introduce artifacts not present in the natural environment. We opted for a natural abundance stable isotopic approach, which provides a powerful tool for tracing N transformations in aquatic systems (Cifuentes et al. 1989; McClelland and Valiela 1998; Middelburg and Nieuwenhuize 2001). To our knowledge, natural abundance stable isotopic ratios have not been used to identify N forms assimilated by phytoplankton.

Nitrogen occurs naturally as two stable isotopes, ^{14}N and ^{15}N , which differ in number of neutrons. The relative abundance of the two forms is denoted as $\delta^{15}\text{N} = [(R_{\text{sample}} : R_{\text{standard}}) - 1] \times 1,000$, where R is the ratio of ^{15}N to ^{14}N (‰). For N, the standard is the ratio of the two isotopes in air. As nitrogen is biologically transformed, fractionation, a change in the ratio of heavy and light isotopes, occurs as a result of the faster reaction rates of molecules containing ^{14}N relative to those with ^{15}N (Kendall 1998). Fractionation is reported as the isotope enrichment factor $\epsilon = [(^{14}\text{N}k / ^{15}\text{N}k) - 1] \times 1,000$, where k is the reaction rate of molecules containing N. A positive value for ϵ means that ^{14}N is used preferentially.

Phytoplankton N uptake causes isotopic fractionation (Table 1) because of preferential assimilation of ^{14}N relative to ^{15}N (Wada and Hattori 1978). The magnitude of fractionation depends on species, light, growth rate, and N concentration (Needoba et al. 2003). The degree of fractionation should be lower with more rapid growth and lower N concentrations (Wada and Hattori 1978). When NH_4^+ is below $20 \mu\text{mol L}^{-1}$, NH_4^+ enters phytoplankton by active transport, and isotopic fractionation is lowest (Fogel and Cifuentes 1993). In contrast to NH_4^+ , the magnitude of fractionation during NO_3^- use is independent of concentration (Pennock et al. 1996). When N is used to depletion, the $\delta^{15}\text{N}$ of the resulting product approaches the original $\delta^{15}\text{N}$ value of the N source.

The range of values reported for fractionation associated with phytoplankton nitrogen uptake is broad but, with one exception, indicates preferential use of the light isotope (Table 1). Laboratory measurements of ϵ for NO_3^- are lower than those for NH_4^+ (-6% to $+16\%$ for NO_3^- use vs. $+15\%$ to $+25\%$ for NH_4^+). Field measurements of ϵ for NH_4^+ and NO_3^- are lower than for laboratory measurements (3% to 9% for NO_3^- and 0% to 14% for NH_4^+ ; Table 1). The lowest values for NH_4^+ fractionation were for phytoplankton under N-starved conditions (Waser et al. 1999), so when NH_4^+ is low or limiting, ϵ can be small.

Many studies have used the $\delta^{15}\text{N}$ of seston to represent the phytoplankton $\delta^{15}\text{N}$ (e.g., Mariotti et al. 1984; Cifuentes et al. 1989; Montoya et al. 1991). This might be

inappropriate because of the diverse mixture of detritus, organic aggregates, and sediment in seston (Fogel and Cifuentes 1993). A recently developed method (Sachs et al. 1999; Sachs and Repeta 2000) makes it possible to determine the $\delta^{15}\text{N}$ of phytoplankton, distinct from the $\delta^{15}\text{N}$ of bulk seston, which could avoid potential contamination from other materials in seston. Chlorophyll *a* (Chl *a*) is isolated from seston then is analyzed for $\delta^{15}\text{N}$.

The $\delta^{15}\text{N}$ of Chl *a* is an ideal indicator of the $\delta^{15}\text{N}$ of phytoplankton for several reasons. First, Chl *a* is a ubiquitous pigment in phytoplankton. Second, it degrades fairly rapidly, so Chl *a* represents live organisms. Finally, although the $\delta^{15}\text{N}$ of Chl *a* differs from that of phytoplankton, it does so consistently. Sachs et al. (1999) determined that the isotopic shift between the Chl *a* molecule and whole phytoplankton cells was $+5.06\% \pm 1.80\%$ (mean \pm SE, calculated from Sachs et al. 1999).

We used a stable isotopic approach to determine forms of N assimilated by estuarine phytoplankton. We determined the concentrations and $\delta^{15}\text{N}$ values of NH_4^+ , NO_3^- , and Chl *a*. We compared the $\delta^{15}\text{N}$ of Chl *a*, as a proxy for phytoplankton, with the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- to determine which form of DIN was assimilated. We collected samples along a salinity gradient in winter, spring, and summer to assess how salinity and season affect phytoplankton N assimilation. Seasonal changes might dramatically alter biogeochemical processing because denitrification (Koike and Sorenson 1988), remineralization of nutrients (Klump and Martens 1983), and algal growth and nutrient uptake are more active at higher temperatures. We collected samples along a salinity gradient to determine whether N processing varied with differing influence of the marine and freshwater end members. Because anthropogenic nutrient loading enters the system via freshwater, collecting samples at different salinities allowed us to trace land-derived N through the estuary.

Methods

Study site—This study was conducted in the estuarine portion of Childs River, a subestuary of the Waquoit Bay system, on Cape Cod, Massachusetts (Fig. 1). Land use on the watershed of Childs River includes a large proportion of residential area, in addition to cranberry bogs, forest, and other land cover types. The mix of land uses results in a land-derived N load to the estuary more than 20 times the N load to a comparable pristine system (Good 2004). Freshwater is delivered to the estuary primarily via groundwater. The watershed is underlain by unconsolidated sands, and estuarine sediments are sand to muddy sand. Childs River water depth averages 1.4 m; maximum depth is 3 m (Valiela et al. 1997). The mean tidal range is ~ 0.5 m. The water column in the uppermost part of the estuary is usually stratified (Geyer 1997).

Water residence time in Childs River varies with prevailing wind direction (Geyer 1997) and other factors. During southwest winds, which are common in summer, residence time is approximately 2.6 d, whereas residence time can decrease to 1 d during offshore winds (Geyer

Table 1. Fractionation factors for various phytoplankton species or assemblages for growth on NO_3^- and NH_4^+ . Species name indicates that the data are from laboratory cultures, whereas location indicates a field study. A positive value means ^{14}N is taken up preferentially.

N form	Species/location	ϵ (‰)	Reference
NO_3^-	<i>Thalassiosira pseudonana</i>	5.3±0.1	Waser et al. 1998a
	<i>Thalassiosira pseudonana</i>	5	Waser et al. 1998b
	<i>Thalassiosira weissflogii</i>	9.7–15.4	Montoya and McCarthy 1995
	<i>Thalassiosira weissflogii</i>	6.2±0.4	Needoba et al. 2003
	<i>Skeletonema costatum</i>	7.5–11.9	Montoya and McCarthy 1995
	<i>Skeletonema costatum</i>	9±0.7	Pennock et al. 1996
	<i>Skeletonema costatum</i>	2.7±0.3	Needoba et al. 2003
	<i>Prorocentrum minimum</i>	2.5±0.3	Needoba et al. 2003
	<i>Isochrysis galbana</i>	2.2–4.4	Montoya and McCarthy 1995
	<i>Isochrysis galbana</i>	3.2±0.4	Needoba et al. 2003
	<i>Pavlova lutheri</i>	–5.7–3.9	Montoya and McCarthy 1995
	<i>Pavlova lutheri</i>	3.6±0.5	Needoba et al. 2003
	<i>Dunaliella tertiolecta</i>	0.7–6.1	Montoya and McCarthy 1995
	<i>Dunaliella tertiolecta</i>	2.2±0.2	Needoba et al. 2003
	<i>Ditylum brightwellii</i>	3.3±0.4	Needoba et al. 2003
	<i>Chroomonas salina</i>	1.2–2.9	Montoya and McCarthy 1995
	<i>Chaetoceros</i> sp.	0.9–4.5	Wada and Hattori 1978
	<i>Chaetoceros simplex</i>	2.7±0.3	Needoba et al. 2003
	<i>Phaeodactylum tricorutum</i>	7.6–16	Wada and Hattori 1978
	<i>Phaeodactylum tricorutum</i>	4.8±0.3	Needoba et al. 2003
	<i>Emiliania huxleyi</i>	4	Waser et al. 1998b
	<i>Emiliania huxleyi</i>	4.5±0.2	Needoba et al. 2003
	<i>Amphidinium carterae</i>	2.2±0.3	Needoba et al. 2003
	<i>Synechococcus</i> sp.	5.4±0.6	Needoba et al. 2003
	North Atlantic	8–9	Altabet et al. 1991
	Equatorial Pacific	2.5	Altabet and Francois 1994
	Equatorial Pacific	5	Altabet 2001
	Southern Ocean	7–11	Altabet and Francois 1994
	Southern Ocean	4–6	Sigman et al. 1999
	Antarctic Polar Front Zone	7.0±1.0	Altabet and Francois 2001
Auke Bay, Alaska	4	Goering et al. 1990	
Bay of Seine, France	4.2±0.8	Savoie et al. 2003	
Chesapeake Bay	7	Horrigan et al. 1990b	
Monterey Bay	5	Altabet et al. 1999	
Baldeggersee, Switzerland	3.0	Teranes and Bernasconi 2000	
NH_4^+	<i>Skeletonema costatum</i>	17.6±7.9	Pennock et al. 1996
	<i>Thalassiosira pseudonana</i>	20±1	Waser et al. 1998a
	<i>Thalassiosira pseudonana</i>	20	Waser et al. 1998b
	<i>Emiliania huxleyi</i>	15–19	Waser et al. 1998b
	<i>Chaetoceros debilis</i>	25	Waser et al. 1998b
	Delaware Estuary	9.1	Cifuentes et al. 1989
	Chesapeake Bay	2.9–7.1	Montoya et al. 1991
	Burrard Inlet, British Columbia	0–14	Waser et al. 1999

1997). Recently, a more detailed analysis of water mass ages was made with radium isotopes (for methodology see Charette et al. 2001), showing that the time for water to travel from salinity 0 to 20 is ~4 d and ranges from <1 d to >10 d (G. Tomasky unpubl. data).

We selected Childs River for several reasons. First, we expected that the relatively high N load to Childs River would result in NH_4^+ and NO_3^- concentrations high enough to allow accurate measurement of their $\delta^{15}\text{N}$ values. Second, we anticipated that the $\delta^{15}\text{N}$ of the two dominant N sources would be measurably different because inputs from septic systems drive the overall N load and

enter the estuary as NO_3^- (Valiela et al. 1992), whereas evidence from isotopic (see the $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ section in Results; York unpubl. data) and other data (Tomasky unpubl. data) suggests that NH_4^+ is supplied primarily by regeneration within the estuary.

Sampling—Sampling was carried out on five dates between September 2001 and July 2003. Fifty liters of water were collected from the top half meter of the water column at each of five stations (Fig. 1) along an estuarine gradient (salinity 0–30), ~2 km long. Water was sieved through a 200- μm filter to remove grazers and large

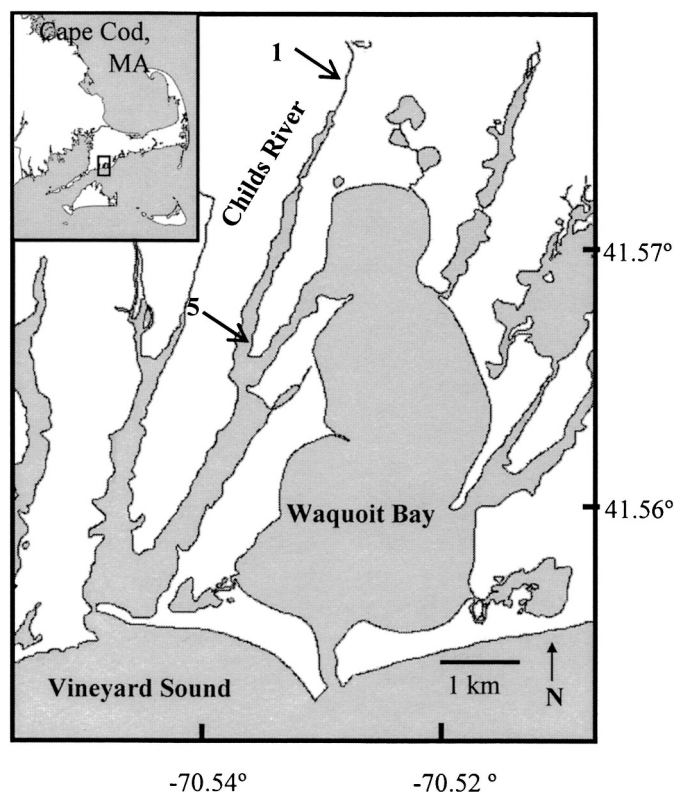


Fig. 1. Waquoit Bay estuarine system and location on Cape Cod, Massachusetts (inset). Sampling was conducted in Childs River between points 1 and 5. Sampling stations varied with salinity but were located at approximately even intervals from salinity 0 to 30.

detritus. We chose sampling stations to represent a regular salinity interval, so the location of stations varied with each sampling date. Salinity was measured with a YSI 95 probe. Water was filtered through precombusted 0.7- μm pore size, 14.7-cm diameter glass fiber filters (TCLP filters, Environmental Express). Depending on seston concentration, as many as 15 filters were used per sampling station. Six liters of the filtrate were reserved for determination of NH_4^+ and NO_3^- concentration and $\delta^{15}\text{N}$. In addition, a small volume (~ 500 mL) of water was passed through a 4.7-cm Whatman GFF filter to measure Chl *a* concentration. All filters and water samples were stored on ice until arrival at the laboratory, when they were transferred to the freezer (-20°C). Samples were generally analyzed within 2 weeks of collection, except those collected for phytoplankton $\delta^{15}\text{N}$ analyses, which took longer to process.

Analyses—Nitrate concentration was determined colorimetrically after cadmium reduction on a Lachat Auto-analyzer. We did not distinguish between nitrate and nitrite, so NO_3^- concentration refers to the sum of the two forms. Ammonium concentration was determined fluorometrically according to the method of Holmes et al. (1999). Chl *a* concentration was determined spectrophotometrically (Lorenzen 1967).

The stable isotopic ratios of NH_4^+ and NO_3^- were determined by diffusion methods (Holmes et al. 1998 and

Sigman et al. 1997, respectively). For the $\delta^{15}\text{N}$ of NH_4^+ , magnesium oxide (MgO) was added to increase pH and convert NH_4^+ to ammonia (NH_3). A filter pack consisting of an acidified glass fiber filter sandwiched between two Teflon filters was placed in each sample bottle. Samples were incubated on a shaker table at 40°C for 14 d, to promote diffusion of NH_3 out of the water and onto the filter pack. To determine the $\delta^{15}\text{N}$ of NO_3^- , samples were initially boiled, with MgO added, to approximately 20% of initial volume to concentrate and drive off NH_4^+ as NH_3 . Devarda's alloy was then added to sample bottles to reduce NO_3^- to NH_4^+ . The rest of the $\delta^{15}\text{NO}_3^-$ procedure continued as for the $\delta^{15}\text{NH}_4^+$ samples. Filter packs from these analyses were analyzed for nitrogen isotopic ratio at the University of California Davis Stable Isotope Facility. Precision of replicate analyses of standards at the U. C. Davis facility was 0.2‰. We generally excluded DIN isotopic data when either NO_3^- or NH_4^+ concentration was below $0.5 \mu\text{mol L}^{-1}$ because determination of isotopic ratios was unreliable at such low concentrations.

Corrections to isotope values were calculated according to Sigman et al. (1997) and Holmes et al. (1998). Standards were analyzed concurrently, with every set of samples analyzed for NO_3^- and NH_4^+ $\delta^{15}\text{N}$. We used differences between the known concentration and nitrogen isotopic composition of standards and the results from the complete analysis (diffusion and mass spectrometer) to make corrections to all samples analyzed in that set. NO_3^- $\delta^{15}\text{N}$ values were also corrected for any additional N added because of N contamination of the Devarda's alloy (Devarda's blanks) as described in Sigman et al. (1997).

Phytoplankton $\delta^{15}\text{N}$ was determined according to Sachs et al. (1999). All filters from a station were composited, and pigments were extracted in 100% acetone by ultrasonication (three times). Pigments were partitioned into hexane (three times) to remove the more polar fraction, then demetalated by treatment with HCl to convert Chl *a* to pheophytin *a*. We used high-pressure liquid chromatography (HPLC) to separate pheophytin *a* from the rest of the pigment mixture. Sample extracts were separated by reversed-phase chromatography (C18 column, Kromasil 100 C18 5 μm , Higgins Analytical) by elution with an acetone-methanol gradient. Pheophytin *a* and pheophytin *a'* were collected and further purified by normal-phase HPLC on a Si column (Supelcosil LC-Si, Supelco) by isocratic elution with 5% acetone/hexane. The pheophytin *a* and pheophytin *a'* fractions were again collected and dried. More details of this method can be found in Sachs et al. (1999). The pheophytin fractions were analyzed for $\delta^{15}\text{N}$ at the Woods Hole Oceanographic Institution gas chromatography-isotope ratio mass spectrometry (GC-IRMS) facility. Analytical precision of these analyses was 0.3‰ on the basis of repeat analyses of nitrogen isotope standards included with each set of samples analyzed on the mass spectrometer. To correct for the difference between the isotopic ratios of Chl *a* and the whole phytoplankton cell, $5.06\text{‰} \pm 1.80\text{‰}$ was added to the value for the Chl *a* (pheophytin *a*) nitrogen isotopic ratio.

To show seasonal differences, we divided the data into summer (July–September) and winter-spring (February and

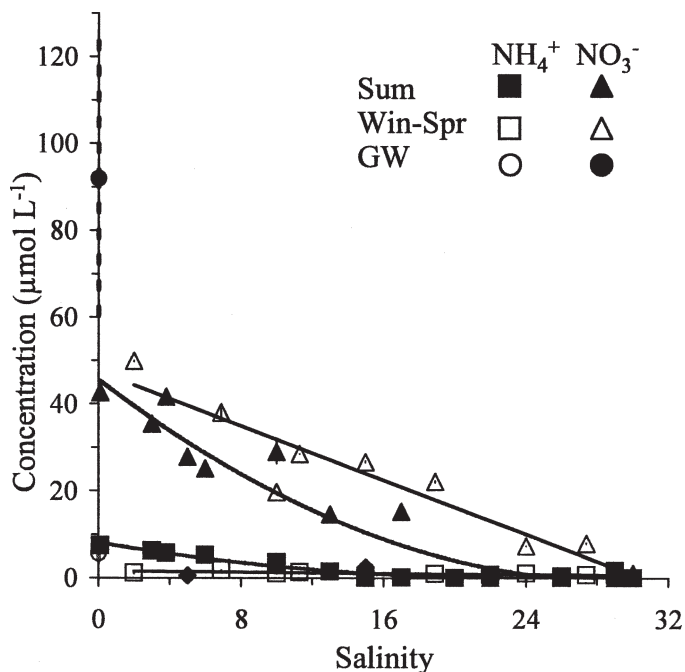


Fig. 2. Concentration of NO_3^- and NH_4^+ relative to salinity in Childs River. Where standard errors are not visible, error was smaller than symbols. Outliers with standardized residuals >2 (Sokal and Rohlf 1995) are denoted by diamonds and were not used to determine regressions. Regression equations and associated statistics are in Table 3. Concentrations of NO_3^- and NH_4^+ in groundwater about to enter Childs River are from Good (2004). Error bars for groundwater concentrations are standard errors and are the heavy dashed lines.

May) for most of the information presented in *Results*. Interannual differences were apparent only for Chl *a* concentrations in summer and likely resulted from different sampling dates between summers (August 2002 vs. July 2003).

Results

Concentrations of NO_3^- and NH_4^+ —The NO_3^- concentration in groundwater entering Childs River was $92.0 \pm 33.6 \mu\text{mol L}^{-1}$ (Good 2004; Fig. 2; Table 2). NO_3^- decreased to $\sim 50 \mu\text{mol L}^{-1}$ in the upper reaches of Childs River where we sampled (Fig. 2), suggesting that roughly half of the NO_3^- in groundwater was lost in the unsampled freshwater upstream reaches or in the seepage zone.

In the estuarine reaches of Childs River during cold months, NO_3^- decreased linearly from ~ 50 to $0 \mu\text{mol L}^{-1}$ (Fig. 2) with salinity, suggesting conservative mixing of freshwater with high NO_3^- with saltwater with low NO_3^- . In contrast, in summer, the downstream decrease in NO_3^- was of the same magnitude but was best described by a concave curve ($r^2 = 0.91$; Table 3), suggesting within-estuary losses of NO_3^- beyond passive mixing.

NH_4^+ concentration followed a similar pattern to that of NO_3^- through the estuary (Fig. 2; Table 2), except that NH_4^+ concentration was almost always lower than NO_3^- . NH_4^+ concentrations were higher in summer than in winter;

the summer maximum was $8.3 \mu\text{mol L}^{-1}$ (at 0 salinity), whereas the winter maximum was $1.8 \mu\text{mol L}^{-1}$ (at 7 salinity). In cool months, estuarine NH_4^+ concentrations were of a similar magnitude as groundwater concentrations. In summer, the possibility of microbial regeneration as a source of NH_4^+ was made evident by concentrations of NH_4^+ at low salinity that were higher than those in groundwater. This seasonal pattern is logical because bacterial activity is greater at higher temperatures (Klump and Martens 1983).

DIN concentrations in Childs River were similar to those in other estuarine systems. In Chesapeake Bay, NO_3^- ranged from 0 to $60 \mu\text{mol L}^{-1}$ and NH_4^+ from 0 to $30 \mu\text{mol L}^{-1}$ (Horrigan et al. 1990a). In the Delaware River estuary, DIN concentrations were higher: NH_4^+ was up to $\sim 70 \mu\text{mol L}^{-1}$ and NO_3^- reached $150 \mu\text{mol L}^{-1}$ (Pennock 1987). By comparison, NO_3^- in Childs River ranged from 0 to $50 \mu\text{mol L}^{-1}$ and NH_4^+ from 0 to $8 \mu\text{mol L}^{-1}$.

Summertime losses of NO_3^- and NH_4^+ beyond passive mixing could have resulted from uptake by primary producers or microbial processes such as nitrification and denitrification. Macroalgae and phytoplankton are both present in Childs River year round and reach peak biomass in summer (Valiela et al. 1997; Hauxwell et al. 1998). Both primary producer groups require N and can draw down DIN in warm months. Nitrification and denitrification are also enhanced at higher temperatures (Koike and Sorensen 1988). In addition, previous work found that denitrification removed 32% to 37% of N entering Childs River and that coupled nitrification–denitrification appeared to be limited by temperature (LaMontagne et al. 2002). Thus, higher temperatures in summer could have promoted DIN loss via nitrification–denitrification during our study.

Phytoplankton biomass—Concentration of phytoplankton Chl *a* depended on season and salinity (Fig. 3; Table 2). The concentration of Chl *a* in winter-spring was relatively low ($1\text{--}15 \text{ mg m}^{-3}$) and did not vary consistently with salinity. In contrast, during summer, Chl *a* increased with salinity from freshwater to midestuary, reaching $\sim 95 \text{ mg m}^{-3}$, then decreased with increasing salinity. A similar midestuarine Chl *a* peak was observed by Cifuentes et al. (1989) in spring in the Delaware River estuary. On one occasion in summer, Chl *a* reached very high levels (116 mg m^{-3}) at low salinity. This might have been the result of extremely favorable growth conditions (temperature, light) coupled with hydrographic conditions resulting in a longer residence time, which allowed proliferation of phytoplankton cells at low salinity.

The bell-shaped pattern of Chl *a* concentration (Fig. 3) was likely the result of growth of phytoplankton during downestuary transport, until a point when DIN became limiting, coupled with subsequent mixing with saltier water with a lower concentration of Chl *a*. In summer, DIN concentrations decreased to zero at salinity > 15 and were probably too low to support high phytoplankton biomass at higher salinity. Summer Chl *a* concentrations in Vineyard Sound (see Fig. 1) were much lower than in Childs River, ranging from 3 to 5 mg m^{-3} (Tomasky et al.

Table 2. Concentrations of NH_4^+ , NO_3^- , and Chl *a*; $\delta^{15}\text{N}$ values of NH_4^+ , NO_3^- , and phytoplankton ($\delta^{15}\text{N}$ -phyto); and calculated values for the percentage of N assimilated by phytoplankton as NH_4^+ ($\% \text{NH}_4^+$) and the fractionation factor for uptake of NH_4^+ . Errors are standard errors, except for $\% \text{NH}_4^+$, which is the standard deviation.

Date	Salinity	NH_4^+ ($\mu\text{mol L}^{-1}$)	NO_3^- ($\mu\text{mol L}^{-1}$)	Chl <i>a</i> (mg m^{-3})	$\delta^{15}\text{N}\text{NH}_4^+$ (‰)	$\delta^{15}\text{N}\text{NO}_3^-$ (‰)	$\delta^{15}\text{N}$ -phyto (‰)	$\% \text{NH}_4^{+*}$	$\epsilon\text{NH}_4^{+\dagger}$
Sep 01									
	3	6.2±0.2	35.5±0.3	3.1±0.9	12.1	6.3	7.1±1.8	63±4	5.0±1.8
	6	5.2±0.0	25.1±0.4	7.3±2.3	10.8	3.1	7.4±1.8	85±4	3.4±1.8
	13	1.4±0.0	14.6±0.2	6.4±1.9	7.2	3.8	9.9±1.8		
	26	0	0	25.7±4.0			8.9±1.8		
	30	0	0	10.6±2.2			6.4±1.8		
Feb 02									
	7	1.8±0.0	38.0±0.1	2.4±0.6	9.0	4.6	3.9±1.8	56±5	5.1±1.8
	11	1.3±0.0	28.5±0.0	4.4±1.1	8.5	1.5	4.2±1.8	75±4	4.3±1.8
	19	1.0±0.0	22.1±0.3	1.1	6.1	1.7	3.9±1.8	76±2	2.3±1.8
	27	0.8±0.1	7.9±0.2	2.8±0.8	2.4	-6.1	6.3±1.8		
	30	0	1.1±0.0	1.3		-7.9	6.0±1.8		
Aug 02									
	4	5.8±0.1	41.6±0.5	6.1±0.7	11.3	6.5	7.7±1.8	77±5	3.6±1.8
	10	3.6±0.0	28.9±2.6	26.2±2.7	12.6	5.4	7.2±1.8	65±3	5.4±1.8
	17	0.2±0.0	15.2±0.5	69.6±8.5	2.9	3.1	7.9±1.8		
	22	0.8±0.6	0	60.0±2.4	2.5		8.5±1.8		
	29	1.7±0.7	0	27.4±0.5	3.8		7.7±1.8		
May 03									
	2	1.1±0.2	49.9±0.1	5.3±0.3	6.8	6.3	6.0±1.8	84±2	0.9±1.8
	10	1.0±0.0	19.7±0.0	3.5±0.1	2.0	2.8	6.4±1.8		
	15	2.5±0.4	26.7±0.1	2.4±0.1	3.9	1.6	6.8±1.8		
	24	1.1±0.0	7.3±0.0	5.2±0.1	-1.6	-2.1	7.0±1.8		
	29	0.8±0.4	0.8±0.0	3.8±0.4	-3.0	-10.1	7.7±1.8		
Jul 03									
	0	8.3±0.1	42.6±0.4	6.3±0.3	13.4	6.9	7.2±1.8	68±7	6.2±1.8
	5	0.6±0.3	27.8±0.1	116.4±11.8	-0.8	5.0	8.1±1.8		
	15	0	0				8.0±1.8		
	20	0	0	95.8±1.1			6.8±1.8		
	26	0.4±0.4	0	22.3±8.0			7.0±1.8		

* Means of calculated results with the use of different fractionation factors for individual samples.

† Calculated as the difference between $\delta^{15}\text{N}\text{NH}_4^+$ and $\delta^{15}\text{N}$ -phyto. Error is the standard error associated with analysis of the $\delta^{15}\text{N}$ of phytoplankton.

1999; Tomasky unpubl. data), which was likely related to low DIN concentrations. In addition, Lawrence et al. (2004) found that copepod abundance in Childs River was highest at salinities ranging from 20 to 30, suggesting that grazing pressure might have also contributed to the decrease in Chl *a* at high salinity.

$\delta^{15}\text{N}$ of NO_3^- and NH_4^+ —The isotopic composition of NO_3^- in Childs River should reflect the end members

contributing NO_3^- to the water, along with fractionation because of processing within the estuary. NO_3^- concentration (Fig. 2) and isotope data (Fig. 4; Table 2) provided evidence that groundwater was the major source of NO_3^- to Childs River. The $\delta^{15}\text{N}$ of NO_3^- in groundwater entering the low-salinity portion of Childs River was $+8.5\text{‰} \pm 5.4\text{‰}$ (McClelland and Valiela 1998). Both concentration and isotopic composition of NO_3^- in groundwater are highly variable (McClelland and Valiela 1998). The combination

Table 3. Statistics for Fig. 2 (NH_4^+ and NO_3^- concentration vs. salinity) and Fig. 4 ($\delta^{15}\text{N}$ of NH_4^+ and NO_3^- vs. salinity). Equations are best fits to the data among a variety of possible curves. n.s., not significant.

N form	Season	Equation	r^2	p
Figure 2				
NH_4^+	Summer	$y=0.02x^2-0.69x+8.10$	0.94	<0.001
	Winter-Spring	$y=-0.03x+1.57$	0.52	n.s.
NO_3^-	Summer	$y=0.05x^2-3.14x+45.58$	0.91	<0.001
	Winter-Spring	$y=-1.56x+47.41$	0.90	<0.001
Figure 4				
NH_4^+	All	$y=-0.42x+11.89$	0.66	<0.001
NO_3^-	All	$y=-0.44x+7.78$	0.87	<0.001

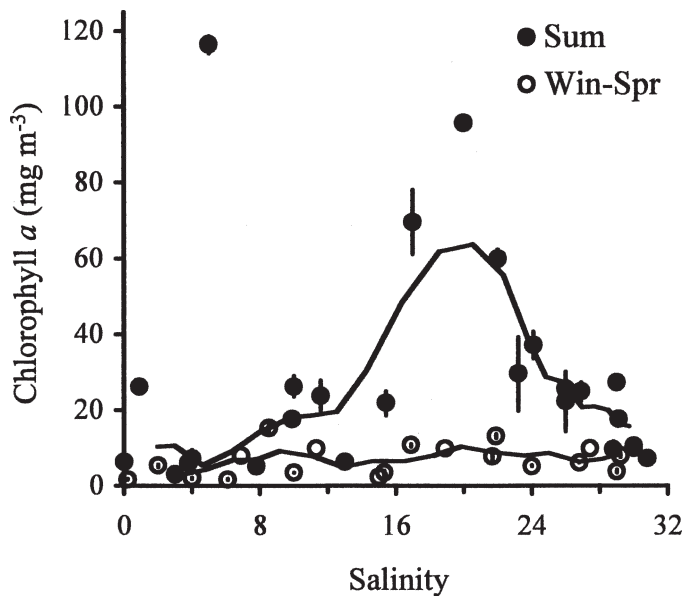


Fig. 3. Chl *a* concentration versus salinity. Where standard errors are not visible, error was smaller than symbols. Samples for Chl *a* concentration were collected on 10 dates (five for this study, plus five additional dates). Lines are four-point running averages.

of concentration data (Fig. 2; Table 2) and the similarity of our $\delta^{15}\text{N}$ value for estuarine NO_3^- (+7‰ at low salinity) with groundwater values made clear the influence of fresh (0 salinity) groundwater NO_3^- inputs. As salinity increased, the $\delta^{15}\text{N}$ of NO_3^- decreased and was lower than the $\delta^{15}\text{N}$ of NH_4^+ throughout the estuary. No difference was detected between summer and winter-spring (analysis of covariance, Table 4).

In contrast to NO_3^- , a comparison of $\delta^{15}\text{N}$ values of NH_4^+ in groundwater and estuarine water suggested that groundwater was not a dominant source of NH_4^+ to the estuary. At low salinity, the $\delta^{15}\text{N}$ of estuarine NH_4^+ was approximately +13‰ (Fig. 4; Table 2). The $\delta^{15}\text{N}$ of groundwater NH_4^+ entering this portion of Childs River was $+6 \pm 3.4\%$ (McClelland and Valiela 1998), indicating that it could not have made a substantial contribution to the estuarine NH_4^+ pool. In contrast, on the basis of incubated sediment cores, York (unpubl. data) found that NH_4^+ regenerated from the benthos had a $\delta^{15}\text{N}$ of $+12.1\% \pm 1.2\%$ in the upper part of the estuary, which more closely matched the estuarine NH_4^+ $\delta^{15}\text{N}$ value (+13‰). The isotopic signal of regenerated NH_4^+ was then “diluted” downestuary, presumably by mixing with NH_4^+ with a lower $\delta^{15}\text{N}$.

Changes in $\delta^{15}\text{N}$ values due to biogeochemical transformations can vary over time or space. The $\delta^{15}\text{N}$ of NH_4^+ varied on a seasonal basis, with higher values in summer relative to winter-spring (Tables 2, 4). This seasonal shift likely resulted from more rapid processing of NH_4^+ in Childs River during warmer months. Concentrations of both NH_4^+ and NO_3^- decreased to zero with increasing salinity (Fig. 2), that is, along a spatial gradient in Childs River. Losses of DIN, beyond conservative mixing, are generally associated with increases in their $\delta^{15}\text{N}$ values

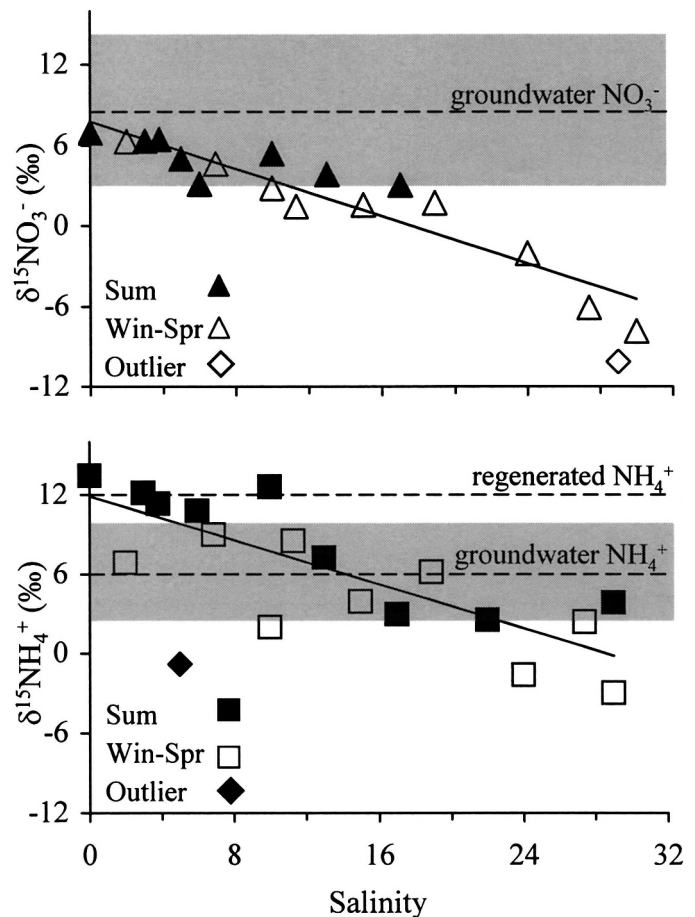


Fig. 4. $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ relative to salinity. Outliers were detected by standardized residuals (value >2 ; Sokal and Rohlf 1995). Regression lines for each were calculated with data from both seasons. Regression equations and related statistics are in Table 3. Summer $\delta^{15}\text{NO}_3^-$ points do not appear because concentration was too low for reliable measurement. $\delta^{15}\text{N}$ of NH_4^+ from regeneration is from York (unpubl. data). $\delta^{15}\text{N}$ of groundwater NO_3^- and NH_4^+ are from McClelland and Valiela (1998). Grey bars represent standard error associated with groundwater values.

because of preferential use of ^{14}N -bearing molecules during phytoplankton uptake, nitrification, denitrification, and other processes (Kendall 1998). We did not, however, observe increases in $\delta^{15}\text{N}$ values typically associated with decreases in DIN on individual sampling dates. A potential

Table 4. Range of $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ for winter-spring and summer samples, including data from 10 sampling dates (five for this study, plus five additional dates). To determine whether $\delta^{15}\text{N}$ of NO_3^- or NH_4^+ was significantly different between summer and winter-spring, we ran an ANCOVA (StatView 5.0.1). n.s., not significant.

	$\delta^{15}\text{NO}_3^-$ (‰)	$\delta^{15}\text{NH}_4^+$ (‰)	Salinity
Winter-spring	+7 to -10	+11 to -3	0-30
Summer	+7 to -9	+13-0	0-29
<i>p</i> value	n.s.	0.03	

explanation could be that increases did occur but were not detected because methodological constraints prevented measurement of $\delta^{15}\text{N}$ values where DIN concentrations were low.

In Childs River, the $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ ranged from -8‰ to $+7\text{‰}$ and -3‰ to $+13\text{‰}$, respectively, and were generally within the range found by others in estuarine systems except for the low NO_3^- $\delta^{15}\text{N}$ values. In the Chesapeake Bay, the $\delta^{15}\text{N}$ of NO_3^- and NH_4^+ ranged from $+4\text{‰}$ to $+11\text{‰}$ and $+3\text{‰}$ to $+21\text{‰}$, respectively (Horrigan et al. 1990b). In the Loire estuary, the $\delta^{15}\text{N}$ of NO_3^- ranged from $+6.5\text{‰}$ to $+20\text{‰}$, whereas NH_4^+ ranged from -4‰ to $+17\text{‰}$ (Middelburg and Nieuwenhuize 2001). The lowest value previously reported for estuarine NO_3^- isotopic ratios was -2.2‰ for the Scheldt estuary (Middelburg and Nieuwenhuize 2001). Several processes could account for our anomalously low NO_3^- $\delta^{15}\text{N}$ values at high salinity, including nitrification and nitrogen fixation; however, additional data would be required to determine whether these processes were important in Childs River.

$\delta^{15}\text{N}$ of phytoplankton—Phytoplankton $\delta^{15}\text{N}$ was relatively invariant with salinity, in contrast to the decrease of $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- with salinity (Fig. 5; Table 2). This pattern suggested that phytoplankton acquired their isotopic ratio upstream then, surprisingly, maintained that $\delta^{15}\text{N}$ during downstream transport. These cells appeared to have internal N stores, acquired by luxury uptake and storage of NO_3^- or NH_4^+ (Dortch 1982; Dortch et al. 1984) upstream. Phytoplankton cultured under N-sufficient conditions can accumulate internal DIN pools of NO_3^- or NH_4^+ up to 90 mmol L^{-1} cell volume (Dortch et al. 1984). Furthermore, stored N can support growth for several days (Dortch et al. 1984). Such a mechanism could explain the relatively unchanging phytoplankton $\delta^{15}\text{N}$ observed downstream in Childs River, although we have no direct evidence that this process occurred. The downstream pattern would suggest that the phytoplankton community that developed between salinity 0 and ~ 12 , before DIN was limiting, was thereafter simply advected downstream and did not change in composition with salinity.

An alternative possibility is that phytoplankton took up urea or dissolved free amino acids (Antia et al. 1991) in addition to DIN. Urea could provide a substantial portion of the nitrogen assimilated by phytoplankton. Antia et al. (1991) found that 20% to 50% of phytoplankton N was taken up as urea by coastal and oceanic phytoplankton. Use of urea was higher when either NH_4^+ was low ($<1 \text{ } \mu\text{mol L}^{-1}$) or where urea concentrations were higher than those of NH_4^+ . There is evidence that uptake of urea is inhibited by the presence of NH_4^+ (Antia et al. 1991; L'Helguen et al. 1996). Although we cannot rule out the possibility that Childs River phytoplankton took up some N as urea or another form, we think its contribution was probably low.

The $\delta^{15}\text{N}$ of phytoplankton varied seasonally, increasing from winter to summer (Fig. 5; Table 2). A paired *t*-test (StatView 5.0.1) showed that differences between winter and spring and between winter and summer were significant ($p < 0.01$ for both comparisons). Phytoplankton

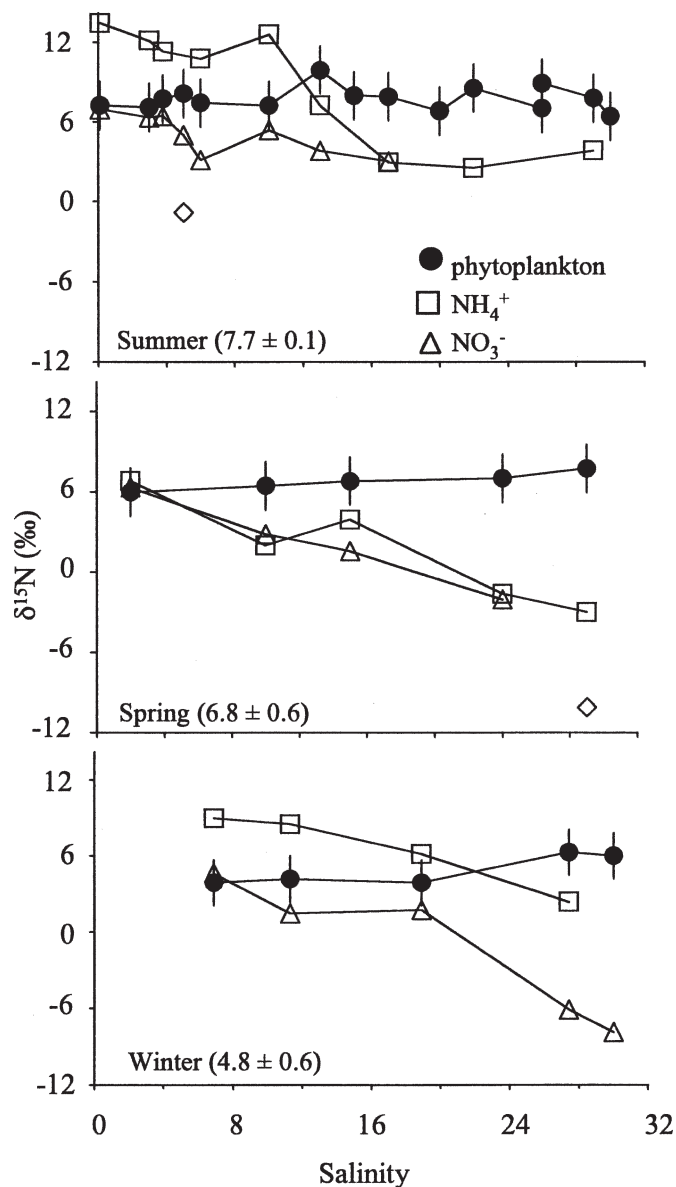


Fig. 5. $\delta^{15}\text{N}$ of phytoplankton, NH_4^+ , and NO_3^- relative to salinity during summer, spring, and winter. Summer NH_4^+ and spring NO_3^- outliers are denoted as diamonds. The mean \pm SE of the $\delta^{15}\text{N}$ of phytoplankton are given in parentheses on each panel.

biomass (as Chl *a*), and presumably growth rate, was also low in winter and increased in warmer months (Fig. 3). Goering et al. (1990) also found that phytoplankton $\delta^{15}\text{N}$ increased with temperature in Auke Bay, Alaska. Mariotti et al. (1984) observed a similar pattern in the $\delta^{15}\text{N}$ of seston in the North Sea. They attributed the summertime increase in $\delta^{15}\text{N}$ to decreased fractionation during rapid phytoplankton growth in warmer months. A similar process might have occurred in Childs River. Alternatively, it is possible that the extent of fractionation did not vary but the $\delta^{15}\text{N}$ of the DIN source increased in warmer months and that this increase was reflected in the $\delta^{15}\text{N}$ of phytoplankton. The $\delta^{15}\text{N}$ of NH_4^+ did increase by $\sim 2\text{‰}$ in warm months relative to cooler months (Table 4). If

phytoplankton used NH_4^+ as their N source, the increase in the $\delta^{15}\text{N}$ of NH_4^+ could have been responsible for the 2.9‰ increase in the $\delta^{15}\text{N}$ of phytoplankton between winter and summer (Fig. 5).

Discussion

Phytoplankton N use—Our original goal was to determine the form(s) of N taken up by phytoplankton by comparison of their stable isotopic ratios. Because phytoplankton use ^{14}N preferentially (Table 1), they become isotopically lighter than their N source. During summer in Childs River, the $\delta^{15}\text{N}$ of phytoplankton was about 7.7‰ and did not vary consistently with salinity, whereas the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- decreased down-estuary (Fig. 5). The main source of N for phytoplankton must have been NH_4^+ , and the only place uptake could have occurred is the upper part of the estuary at salinities between 0 and 12. The $\delta^{15}\text{N}$ of NO_3^- was lower than that of phytoplankton, so it seems unlikely that phytoplankton assimilated NO_3^- . In summer, DIN concentrations dropped to zero at relatively low salinity (Table 2; Fig. 2); July concentrations of NH_4^+ and NO_3^- decreased to zero between salinity 5 and 15 (Table 2), although Chl *a* concentrations continued increasing up to salinity 20, suggesting that phytoplankton growth was fueled by N derived further upstream. The same general pattern was observed in spring and winter, although some NO_3^- use was possible at the lowest salinities in spring and winter. These results suggest a surprising picture of phytoplankton dynamics in Childs River. It seems that phytoplankton acquired a significant pool of NH_4^+ early in their passage through Childs River that supported further growth and division during transport down-estuary.

Visual interpretation of Fig. 5 suggests that NH_4^+ was the dominant N source for phytoplankton, but it is also possible to calculate the relative contribution of NH_4^+ and NO_3^- to phytoplankton N needs. On the basis of the isotopic ratios of NO_3^- , NH_4^+ , and phytoplankton and of the fractionation factors associated with DIN use, it is possible to back-calculate the proportion of the two N forms used, assuming no preferences. We determined the percent contribution of two sources of N to one sink by applying the following equation (Shearer and Kohl 1993),

$$\%A = 100 \times [(\delta^{15}\text{N}_B - \varepsilon_B) - \delta^{15}\text{N}_{\text{sink}}] / [(\delta^{15}\text{N}_B - \varepsilon_B) - (\delta^{15}\text{N}_A - \varepsilon_A)]$$

where A is NH_4^+ , B is NO_3^- , and phytoplankton is the sink; $\delta^{15}\text{N}_B$ is the $\delta^{15}\text{N}$ of NO_3^- , ε_B is the fractionation factor for NO_3^- use, $\delta^{15}\text{N}_{\text{sink}}$ is the $\delta^{15}\text{N}$ of phytoplankton, $\delta^{15}\text{N}_A$ is the $\delta^{15}\text{N}$ of NH_4^+ , and ε_A is the fractionation factor for NH_4^+ use.

We measured the $\delta^{15}\text{N}$ of NH_4^+ , NO_3^- , and phytoplankton and obtained estimates of fractionation factors from the literature (Table 1), which yielded a range of ε values. We opted to use values for ε that were determined for natural phytoplankton assemblages from coastal or estuarine locations. We solved the above equation using our

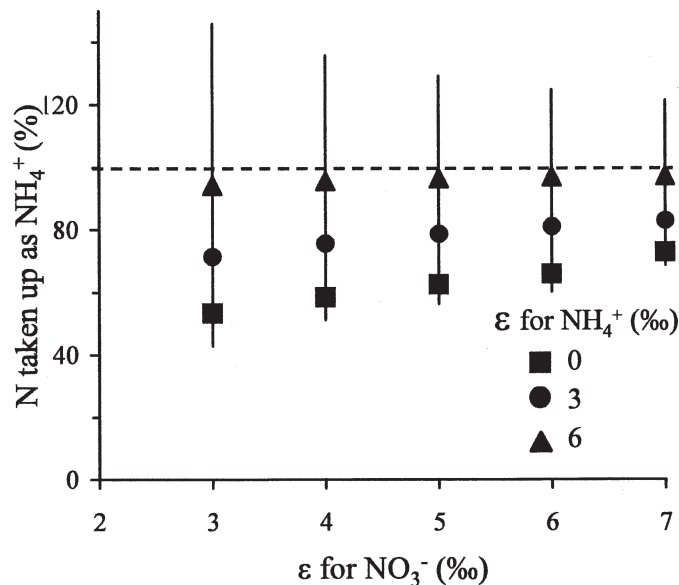


Fig. 6. Percentage of N taken up by phytoplankton as NH_4^+ as a function of different values of ε for NO_3^- (x-axis) and NH_4^+ (different symbols). NO_3^- fractionation factors were 3‰, 4‰, 5‰, 6‰, and 7‰ and NH_4^+ fractionation factors were 0‰, 3‰, 6‰, 10‰, and 14‰. Symbols for 10‰ and 14‰ do not appear on the plot because solutions to the mixing equation using these estimates were not between 0% and 100%. Error bars indicate propagated standard error for % NH_4^+ from error associated with the $\delta^{15}\text{N}$ of phytoplankton (± 1.80 ‰; for equation see the *Phytoplankton N use* section in *Discussion*) and averaging of solutions to that equation on the basis of different combinations of fractionation factors. Error bars for $\varepsilon_{\text{NH}_4^+} = 6$ ‰ overlap with other error bars. Dashed line delineates 100% of N taken up as NH_4^+ .

data for the isotopic ratios and varied ε for NO_3^- from 3‰ to 7‰ and ε for NH_4^+ from 0‰ to 14‰.

Because phytoplankton must become isotopically lighter than their N source, we only calculated solutions to the above equation for occasions when the $\delta^{15}\text{N}$ of either NO_3^- or NH_4^+ was higher than that of phytoplankton. This restricted our calculations to 9 of the 25 samples collected, and the salinity range to 0–19. Use of our chosen values for fractionation factors (NO_3^- , 3‰–7‰; NH_4^+ , 0‰–14‰) resulted in some solutions that were unreasonable (i.e., >100% or negative values). In Fig. 6, we only show solutions between 0% and 100%.

We found, on the basis of mean values calculated for combinations of values for $\varepsilon_{\text{NO}_3^-}$ and $\varepsilon_{\text{NH}_4^+}$, that phytoplankton assimilated from 53% to 97% of their N as NH_4^+ (Fig. 6). If we instead calculated means for individual phytoplankton samples, the range of values for the percentage of N taken up as NH_4^+ was 56–85% (Table 2). Pennock (1987) also found that NH_4^+ provided the majority of N for phytoplankton in the Delaware River estuary, where NO_3^- concentrations were greater than NH_4^+ concentrations. The amount of N that phytoplankton took up as NH_4^+ and the NH_4^+ concentration did not show a clear relationship, suggesting that NH_4^+ availability did not affect the relative proportion of NH_4^+ and NO_3^- taken up by phytoplankton. It seems that regeneration

maintained NH_4^+ concentrations in the estuary at levels high enough to support phytoplankton growth.

Fractionation factor for phytoplankton NH_4^+ use—On the basis of the assumption that phytoplankton generally assimilated NH_4^+ in Childs River, we calculated a fractionation factor associated with this process. We took the mean of the difference between the $\delta^{15}\text{N}$ of NH_4^+ and phytoplankton for those samples where $\delta^{15}\text{NH}_4^+ > \delta^{15}\text{N}$ -phytoplankton, which included samples from salinity 0–19. This value could represent a low estimate because any use of NO_3^- would result in a decrease in the $\delta^{15}\text{N}$ value of phytoplankton and the relative difference between the $\delta^{15}\text{N}$ of NH_4^+ and phytoplankton would therefore be greater. The calculated value $\epsilon = 4.0\text{‰} \pm 0.6\text{‰}$ is in the lower range of reported fractionation factors (Table 1), which might indicate that Childs River phytoplankton were N limited, resulting in a relatively small value for ϵ . Tomasky et al. (1999) found that in spite of high DIN in Childs River, N supply limited phytoplankton growth at salinity as low as 10. Waser et al. (1999) found that the fractionation factor was 0‰ for phytoplankton growth on NH_4^+ under simulated bloom conditions when phytoplankton had been N starved.

Implications—Eutrophication of coastal waters is increasing and is driven primarily by increased delivery of NO_3^- (Vitousek et al. 1997; Cloern 2001). The cascade of effects associated with eutrophication is well documented and includes stimulation of primary producers, including phytoplankton. Childs River, where we conducted our study, receives a large nitrate-dominated N load and has NO_3^- concentrations up to 40 times higher than NH_4^+ concentrations. Phytoplankton apparently respond to the increased N load; Chl *a* concentrations are significantly higher than in other similar nearby estuaries, with lower N load and NO_3^- concentrations (Valiela et al. 1992). Our results suggest the paradox that high NO_3^- concentrations clearly lead to eutrophic conditions, but it seems that phytoplankton derive the majority of their N as NH_4^+ , indicating that the mechanism by which NO_3^- contributes to eutrophication might be indirect.

Method assessment—Our method offers an alternative to traditional ^{15}N uptake methods (Dugdale and Wilkerson 1986) to estimate assimilation of nitrogen by phytoplankton. A major benefit of our method is that it avoids concerns associated with bottle incubations, which could be particularly advantageous in oligotrophic waters in which longer incubation times could be required for ^{15}N uptake experiments. Our method further provides an integrated assessment of phytoplankton N use up to the time of sampling, whereas the ^{15}N method provides information on the form of N taken up at the time of incubation. Disadvantages of our method include the uncertainty introduced by the relatively poorly constrained values for ϵ associated with N assimilation and that it does not consider urea. It might be possible to experimentally determine ϵ for the phytoplankton community, which would clarify some concerns. In addition, it is possible to determine the $\delta^{15}\text{N}$ of urea (Rysgaard and Risgaard-

Petersen 1997), so this N source could be incorporated into the method. As with any natural abundance stable isotopic study, care must be taken in choosing an appropriate study site. For this method, concentrations of DIN and phytoplankton must be high enough to allow measurement of their $\delta^{15}\text{N}$ values. Recent advances in analytical methods (e.g., the denitrifier method for measurement of $\text{NO}_3^- \delta^{15}\text{N}$; Sigman et al. 2001) would minimize this concern. In addition, the $\delta^{15}\text{N}$ values of different N sources should differ enough to ensure that it is possible to distinguish their signals in the $\delta^{15}\text{N}$ of the phytoplankton. This method is ideal for use in concert with broad surveys of stable isotopic values as, for example, in food web studies.

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Received: 10 December 2005

Accepted: 8 June 2006

Amended: 28 July 2006